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Trace elements and rat pouchitis

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The procedure of restorative proctocolectomy is associated with a complete removal of the colon and slight reduction of ileum length, which together can lead to systemic shortages of trace elements. Inflammatory changes in the pouch mucosa may also have some impact. However, there is no data on trace elements in pouchitis. Therefore, in the present study we aimed to assess the effect of acute pouchitis on the status of selected trace elements in rats. Restorative proctocolectomy with the construction of intestinal J-pouch was performed in twenty-four Wistar rats. Three weeks after the surgery, pouchitis was induced. Eight untreated rats created the control group. Liver concentrations of selected micronutrients (Zn, Cu, Co, Mn, Se) were measured in both groups six weeks later, using inductively coupled plasma mass spectrometry. Liver concentrations of trace elements did not differ between the study and the control groups. However, copper, cobalt and selenium concentrations [µg/g] were statistically lower (*p*<0.02, *p*<0.05 and *p*<0.04, respectively) in rats with severe pouchitis (n=9) as compared with rats with mild pouchitis (n=7) [median (range): Cu — 7.05 (3.02–14.57) *vs* 10.47 (5.16–14.97); Co — 0.55 (0.37–0.96) *vs* 0.61 (0.52–0.86); Se — 1.17 (0.69–1.54) *vs* 1.18 (0.29–1.91)]. In conclusion, it seems that acute pouchitis can lead to a significant deficiency of trace elements.

Key words: restorative proctocolectomy, pouchitis, trace elements, selective deficiency

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INTRODUCTION & AIM

Trace elements are a large group of chemical elements present in living matter in concentrations below 1000 ppm (< 0.1%). This group of over 60 elements demonstrates very different biochemical properties. Body resources of trace elements depend not only on an adequate supply in the diet, but also on their proper absorption and excretion (Sandström *et al.*, 2001; Serra-Majem L *et al.*, 2009). Pathological changes in morphology and functioning of the gastrointestinal tract can compromise trace element absorption (Sandström *et al.*, 2001, Berdanier, 2004). A potential example of such an effect could be inflammation of the intestinal reservoir (pouchitis) appearing in many patients who underwent restorative proctocolectomy. During the procedure, the whole colon is removed, and the length of the small intestine is slightly reduced (and thus the total surface absorption). In addition, the presence of bloody, loose stools (that can be correlated with severe pouchitis) predisposes these patients to deficiencies of iron as well as other nutrients (Yu *et al.*, 2007). There are only few studies assessing the status of selected trace elements in patients with pouch and pouchitis.

Iron and calcium deficiencies in patients with pouch and pouchitis are quite common. However, there is no comprehensive data describing the status of other elements (M'Koma *et al.*, 1994; Kuisma *et al.*, 2001; Pastrana *et al.*, 2007). In the only published study, copper and selenium concentrations in patients with pouch did not differ significantly from those in healthy subjects (El Muhtaseb *et al.*, 2007). Similarly, the daily intake of those elements in the diet was comparable to that documented in the control group. It should be emphasized that the evaluation of trace element status was based on serum levels, which do not reflect the real body resources. It seems that the content in the liver, being the storage organ, better reflects the long-term effects. However, there is no data on the trace element status in pouchitis. Therefore, in the present study we aimed to assess the effect of pouchitis on the status of selected micronutrients in rats. To increase the reliability of the assessment we measured liver concentrations of microelements.

MATERIAL & METHODS

Restorative proctocolectomy with the construction of intestinal reservoir was performed in twenty-four Wistar rats (study group). The total proctocolectomy was performed by resecting of the colon and ligating the mesentery with 4–0 silk. The intestinal segment was excised from 0.1 cm proximal to the ileocecal junction. The rectum was resected at the level of the pelvic floor, with a 0.5 cm rectal stump. The ileal J-pouch was created by the duplication of the distal end of the small intestine by a single-layer interrupted 6–0 prolene suture. The pouch anal anastomosis was performed with a single layer interrupted 6–0 prolene suture (Babu *et al.*, 2005). Additional, eight rats (which did not undergo any surgery) created the control group.

After the first fasting day (with exclusive supply of 8% glucose solution), the animals from the study group received fiber-free, semi-synthetic AIN-93 diet, in increasing amounts in subsequent 10 days (respectively from 5, 8, 10 and 12 g/d up to 25 g/d). The feeding of rats was

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Abbreviations: ICP-MS, inductively coupled plasma mass spectrometry.

Table 1. Liver concentrations of trace elements in the study and the control group

Trace element	Liver concentrations [μg/g] Median (Range)	
	Study group	Control group
Zn	89.36 (33.39–187.60)	86.76 (42.07–217.10)
Cu	9.22 (3.02–14.97)	10.54 (4.15–16.56)
Co	0.59 (0.37–0.96)	0.63 (0.56–0.90)
Mn	8.02 (4.43–9.70)	9.02 (6.35–12.63)
Se	1.24 (0.29–1.91)	1.27 (1.22–1.68)

maintained at the 25 g/d level for 11 following days. Subsequently, inflammation of J-pouch was induced following a procedure developed in our previous studies (Drzymała-Czyż *et al.*, 2012). For that purpose, the animals were given fiber-enriched AIN-93 diet for seven days (with growing fiber quantities, 1% on the first day, 2% on the second, up to a maximum of 4% of the content). The control group was fed *ad libitum* AIN-93 diet, supplemented for a period of seven days with fiber (at the same time as in the study group). Over the next six weeks, the animals from both groups were fed *ad libitum* semisynthetic AIN-93 diet. After the scheduled feeding period the animals were euthanized and specimens of J-pouch mucosa for the histopathological and immunohistochemical analysis were obtained. Liver concentrations of trace elements were assessed in all animals.

Toxicological examination. After determining the dry weight of samples from liver biopsies, the mineralization was carried out following rehydration with concentrated 65% HNO_3 (Supapur, Merck, Darmstadt, Germany). The levels of trace elements in prepared samples were determined using inductively coupled plasma mass spectrometry (ICP-MS; camera: ELAN DRC, Perkin Elmer, Waltham, Massachusetts, USA) (Olivares, 1998). ICP-MS is a technique which is used to measure the intensity of an ion flux generated in the plasma. Ions generated inductively in the coupling plasma are then distributed through the mass analyzer based on the amplitude of their weight relative to their charge. Samples are analyzed in a liquid form by introducing them into a system consisting of a spray and fog chamber.

Histopathological examination. Microscopic assessment was performed according to standard histological techniques (hematoxylin and eosin staining). In addition to routine histopathological examination, the collected specimens were evaluated for the intensity of inflammation (Moskowitz scale) (Sandborn *et al.*, 1994). Based on the results of this examination rats were divided in to two subgroups – with acute (inflammation assessed to 4–6 in Moskowitz scale; n = 9) and mild pouchitis (Moskowitz 1–3; n = 7).

Statistical analysis. For the results obtained, medians and ranges of values are given. The Mann-Whitney test was used to compare the results between the study groups. The hypotheses were verified at a 0.05 significance level. Correlations were evaluated using the Spearman test.

Ethical considerations. All surgical procedures were conducted by one qualified surgeon in accordance with the guidelines of the European Community Council directive 86/609/EEC and with the approval of the Local Ethics Committee (42/2006).

Table 2. Liver concentrations of trace elements in rats with severe and mild pouchitis

Trace element	Liver concentrations [μg/g] Median (Range)		
	Severe pouchitis	Mild pouchitis	Control group
Zn	82.85 (33.39–134.70)	95.81 (67.52–187.60)	86.76 (42.07–217.10)
Cu	7.05[a,b] (3.02–14.57)	10.47[a] (5.16–14.97)	10.54[b] (4.15–16.56)
Co	0.55[b,c] (0.37–0.96)	0.61[c] (0.52–0.86)	0.63[b] (0.56–0.90)
Mn	7.80 (4.43–9.59)	8.71 (6.94–9.70)	9.02 (6.35–12.63)
Se	1.17[b,d] (0.69–1.54)	1.18[b] (0.29–1.91)	1.27[d] (1.22–1.68)

[a]$p<0.02$; [b]$p<0.04$; [c]$p<0.05$; [d]$p<0.005$

RESULTS

The liver concentrations of trace elements in the study and control groups are summarized in Table 1. For elements tested no statistically significant differences were stated.

The liver concentrations of trace elements depending on the severity of inflammation (expressed in the Moskowitz scale) are presented in Table 2. Copper, cobalt and selenium concentrations were statistically lower in rats with severe pouchitis as compared with rats with mild pouchitis and the control group.

The liver concentrations of trace elements did not correlate with the severity of inflammation expressed in the Moskowitz scale.

DISCUSSION & CONCLUSIONS

In the present study, liver concetrations of zinc, copper, cobalt, manganese and selenium did not differ significantly between rats with J-pouch and a control group. Bones and muscle are significant reservoirs of trace elements, but their hightest concentration is present specifically in the liver (Sandström *et al.*, 2001; Berdanier, 2004; Serra-Majem L *et al.*, 2009). Therefore, liver seems to be the most appropriate organ that best reflects the body resources of these micronutrients.

El Muhtaseb and coworkers (2007) documented that plasma concentrations of zinc, copper and selenium, as well as their intake in the diet of patients with pouch (n=55) did not differ significantly from those observed in healthy subjects (n = 46), although the procedure of restorative proctocolectomy involves a complete removal of the large intestine and a reduction in the length of the ileum, which can affect the efficiency of nutrient absorption. Coexisting inflammation can additionally contribute to the intensity of possible abnormalities. On the other hand, El Muhtaseb and coworkers documented that the plasma concentration of manganese was higher in patients who underwent restorative proctocolectomy than in those from the control group. The symptoms and consequences arising from excess (overload) manganese are not completely understood; however, they are seen mostly in the course of parenteral nutrition (Arjona *et al.*, 1997; Bertinet *et al*., 2000). The authors attributed the excessive manganese levels to the use of antidiarrheal drugs containing this trace element and to the coexisting iron deficiency, which in this case could encourage the excessive absorption of manganese. It should be emphasized that these results cannot be directly compared with our data. None of the patients involved in the El Muhtaseb *et al.* (2007) study showed any clinical symptoms of pouchitis, their inflammatory markers (CRP) were negative and the use of antidiarrheal drugs was common. The assessment of microelement status (Zn, Cu, Mn, Se) was based on plasma concentrations which cannot create the basis for a definite assessment of their body resources. In the present study, rats were suffering from significant pouchitis, while trace element assessments were based on liver tissue samples.

M'Koma and coworkers (1994) noted that 2–5% of patients post ileal pouch–anal anastomosis had low zinc levels. However, Pironi *et al.* (1991) documented low serum zinc levels in 60% of patients with ileal pouch. Those authors attributed the lowered zinc levels to greater requirements of that trace element and secondarily to increased muscle protein synthesis. For the purpose of the study, they evaluated 18 patients witch pouch. Interestingly, although the prevalence of hypozincemia was very high, pouch enteritis according to approved criteria (the presence of diarrhoea accompanied by endoscopic features of acute inflammation and by histological evidence of a prominent polymorphonuclear cell exudate), was observed only in 2 patients.

Liver levels of copper, cobalt and selenium in the subgroup with severe pouchitis were lower than in the subgroup with mild pouchitis. There is no published data concerning the body resources of these trace elements in patients or animals with severe pouchitis. The assessment of trace element status was conducted in patients with ulcerative colitis (clinically most similar to the model of pouchitis), serum zinc levels were lower and serum copper levels higher in the active colitis group than in controls (Ringstad *et al*., 1993). More than 50% of patients with active form of ulcerative colitis showed zinc levels below the 15th percentile of the control group. It should be noted that in spite of the decrease in disease activity, serum zinc levels remained low also after introduction of total enteral nutrition. In contrast, Vagianos *et al.* (2007) documented that the prevalence of subnormal serum zinc levels in 126 adults patients with ulcerative colitis was not high — 12.5%. Moreover, the median serum level of zinc was comparable in subjects with active ulcerative colitis (assessed using Powell-Tuck Index) and subjects in remission.

The obtained results suggest that not restorative proctocolectomy itself but acute pouchitis can lead to a significant deficiency of trace elements. Therefore, specific human studies should be performed. It seems that in future in selected groups of patients with pouchitis supplementation of trace elements should be considered.

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All authors declare no conflict of interest.

Author's contribution

SDC — study design, data collection, analysis of samples, statistical analysis, data interpretation, manuscript preparation, literature search. TB — study design, data collection, data interpretation, manuscript preparation, literature search. SW & EWC — analysis of samples, data interpretation. TK & MD — study design, data interpretation. JW — study design, statistical analysis, data interpretation, manuscript preparation, literature search.

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